Noteworthy anatomical and physiological researches.

Researches on transpiration and assimilation.[1]

I. *Transpiration experiments.*

Stahl's purpose in writing the paper here reviewed was mainly to present a method by means of which it could be demonstrated to the eye whether a plant loses water by transpiration, and through what parts of its surface the loss takes place. The method does not take the place of weighing the loss of water for all the more accurate experiments. It is valuable on account of its simplicity and the facility it offers for public demonstration coupled with the fact that by it certain problems can be investigated which are not possible by other methods.

Marget[2] was probably the first investigator who used the method about to be described. According to Stahl he impregnated white paper with a mixture of mercurous chloride and pallidous chloride, and also with pallidous chloride, tartaric acid and ferrous chloride. In the dry state the paper was whitish-yellow, but as it absorbed water it became darker and finally black. When applied to the transpiring surface the loss of water could be estimated by the change in color. The color could be fixed at any point by wetting the paper in ferric chloride.[3] Others who have tried to use Marget's

[1] Stahl, Ernest. Einige Versuche über Transpiration und Assimilation. Bot. Zeit. **52:** 117–145. *pl. 4.* Jl. 1894.

[2] Marget, A. Sur les fonctions des feuilles. Rôle des stomates dans l'exhalation et dans l'inhalation des vapeurs aqueuses par les feuilles. Comptes-rendus de l'acad. des sc. **87:** 293. 1878.

Recherches sur la transpiration des végétaux et le rôle des feuilles dans ce phénomène. Annales de la soc. d'Agric. etc. de Lyon **1878:** LXXV–LXXVII.

[3] The first account of this method published by Marget in Annales de la Soc. d'Agric. etc. de Lyon l. c. says that the paper was treated with a solution of pallidous chloride, tartaric acid and ferric chloride, *not ferrous chloride.* The paper was then dried and exposed to light which changed the ferric chloride to ferrous chloride. In this condition the paper when moistened turned darker and finally black as the amount of moisture increased. In the last account, Comptes rendus l. c., he simply says that he used a paper sensitized photochemically with a mixture of pallidous chloride and ferrous chloride. The method of doing this is not given but is probably the same as that given in the first paper. The changes in color are given and also a method of fixing the color at any point by simply soaking the exposed paper in a solution of ferric chloride. It is possible with the corrections here indicated that the method may succeed, though I have not had opportunity to test it. It would certainly have advantages over the cobalt method if it could be used readily. I find no mention in the papers cited of the use of mercurous chloride.

method have failed, probably because it was so briefly described by him. Stahl found a much more satisfactory material in the cobalt salts, especially cobalt chloride.[4] Strips of Swedish filter paper were soaked in from 1–5 per cent. water solution of this salt and then dried. When dry the color varies from a light blue in the 1 per cent. solution to a deeper blue in the stronger solutions. The color fades from very light blue to pink, then white, as the paper becomes moist. The paper impregnated with the stronger solution is best for public demonstration but that soaked in the one per cent. solution is best for comparing small differences in evaporation from various parts of the same leaf. A small piece of cobalt paper having been thoroughly dried, is placed on the part of the plant desired, then to hold it firmly against the surface and protect it from the air a thin piece of glass or mica is placed over it and held on with small clamps. After the observation is made the piece may be dried ready for use again.[5]

The author describes a number of experiments with this paper as an indicator which I will briefly summarize.

1. *Stomatic and cuticular transpiration.*—In cases where leaves have stomata only on the under side this side reddens the cobalt paper very quickly, often in a few seconds, while paper on the upper side of the same leaf holds its color often for several hours. The contrast between the upper and under surface is present even in the smallest leaves accessible to treatment, even those still in vernation. Among the plants used are *Syringa vulgaris*, *Cornus stolonifera*, *Ribes alpinum*, *Populus fastigiata*, and *Quercus pedunculata*. Where stomata occur on both sides the reddening of the paper is proportional to the number. In *Trifolium repens*, which has more stomata on the upper than on the under side, the paper faded most rapidly on that side. In the case of slightly wilted

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[4] Cobaltous chloride was used by Marget but abandoned because the change in color passes quickly and can not be fixed. See Annales de la Soc. de Agric. etc. de Lyon l. c.

[5] In using the paper I have found it necessary to exercise caution in regard to several points. Any soft filter paper that takes up moisture readily may be used. Care should be taken to wet the paper evenly with the cobalt. Strips of paper may be dipped in the solutions then laid on blotting paper to absorb the extra solution and dried either in a warm oven or over sulphuric acid. Care must be taken that the cobalt paper is pressed with equal force at all points against the surface to be tested, otherwise the parts most closely pressed against the plant will fade first. This is particularly liable to occur where mica is used to protect the paper from the air.

leaves and in fact in all cases where the stomata were closed on account of an insufficiency of water in the plant even though they were exposed to the sun, no reddening of the paper occurred. This shows how very small the evaporation is through cuticularized epidermis even when exposed to the bright sun. The same was found to be true with fully turgescent leaves supplied with water.

2. *Regulation of transpiration by means of the guard-cells.*—Stahl confirms the observation of Mohl, Leitgeb and others that in the wilting of a leaf the guard-cells are the first to be affected by the loss of water. When two leaves as nearly alike as possible except that one is slightly wilted and has the stomata closed, are placed together between two pieces of glass and the upper surfaces exposed to the bright July sun, the one with the stomata closed gives off no water and wilts no further while the turgid one with open stomata at the start discolors the cobalt paper rapidly and becomes completely wilted. This was found true in all cases investigated, viz., leaves of *Tropaeolum majus*, *Tradescantia zebrina*, *Pharbitis hispida*, *Pelargonium zonale*, *Rhus cotinus*. Rapid wilting also occurs when freshly picked leaves are exposed to bright sun in highly saturated air, while leaves which were first slightly wilted so that their stomata are closed lose no more water when exposed to the sun. Sometimes the stomata would open again in the saturated air and then transpiration would continue.

These experiments show that stomata do not close in saturated air even though the evaporation from the leaf has led to its complete wilting. They further indicate that as the stomata open widest in bright sunlight and saturated air that evaporation under these conditions may be much greater than we expect. Haberlandt's observations on tropical plants[6] (viz., that they lose two or three times less water than plants in Germany) may be even very short of the reality for plants removed from the direct influence of the sun. On the other hand those exposed to the direct sun probably lose more than Haberlandt found. Stahl found that many marsh plants and shrubs in the damp tropical forests are unable to close their stomata. When exposed to dry air they dry up in a few hours. It was also found in these cases that the loss was

[6] Haberlandt, G. Anatomisch-physiologische Untersuchungen über das tropische Laubblatt. Sitzungsb. d. Wiener Akademie **101**: 785–816. O. 1892.

mostly through the stomata. A number of trees inhabiting damp soil were also found to be unable to regulate transpiration, viz., *Betula alba*, *Alnus glutinosa*, and various species of *Salix;* also the shrub *Hydrangea hortensis*, so thoroughly examined by Wiesner.[7]

"The ability to close the stomata, however, under conditions unfavorable for assimilation is surely very common with plants, which in their native locality frequently have to battle with a transient scarcity of water." An hermetic closing of the stomata is especially common with plants whose vegetation is interrupted by long periods of rest. Cobalt tests made October 20th showed a complete closing up of the stomata in *Buxus sempervirens*, *Mahonia aquifolium*, and *Taxus baccata*. They were still open in *Ilex aquifolium* and *Hedera helix*.

Evergreen leaves which have discontinued stomatory transpiration will not redden the cobalt paper even after they have been exposed for a long time in a hot moist room to the sun. For example vigorous branches of Buxus, Mahonia, Ilex, Hedera, and Taxus were cut off in sunny frosty weather Dec. 12th. The cut surfaces were immersed in water and the branches exposed in a moist atmosphere to the sun. The cobalt test after three hours showed a loss of water only in *Ilex aquifolium*. After eight days Taxus and Mahonia began to show loss but Buxus and Hedera still kept their stomata closed. Of all these evergreens, Ilex is the one that suffers first from the cold. Stahl confirms the earlier observations that in colored autumn leaves the stomata are closed. He agrees with Wiesner that the reduction in transpiration greatly influences the defoliation of deciduous plants.

II. *Role of the stomata in the exchange of gases during assimilation.*

On account of the ease with which the cobalt test shows whether the stomata are open or closed, it is a valuable aid in the study of assimilation and exchange of gases in the plant. Stahl proceeded to throw more light on the question as to whether under the usual conditions occurring in nature there was sufficient exchange of gases through the cuticularized epidermis of land plants to render possible the assimilative activity necessary for their growth.

[7] Sitzungsber. der Kais. Akad. d. Wiss. in Wien. **96:** 182–214. Je.–D. 1887.

Kreusler[8] has shown the great influence of the amount of water contained in leaves on their assimilative activity. "While a complete saturation of the atmosphere does not in itself seem unfavorable to assimilation, the latter may, on the other hand, be considerably reduced by a dry atmosphere and the subsequent powerful evaporation, long before the leaf loses visibly in turgescence. Although it is well known that starch does not represent the primary product of assimilation[9] still its presence or absence may be used as a good comparative test. It was found that in thase plants which can not close their stomata even after they are quite withered starch was made, viz., in *Rumex aquaticus*, *Caltha palustris*, *Hydrangea hortensis*, and *Calla palustris*. On the other hand leaves with closed stomata made no starch. If the stomata on a turgid leaf are closed with a mixture of one part bleached bees-wax and three parts of cocoa butter (this mixture is perfectly harmless to the leaf, does not melt below 40° C. and is easily washed off with water) no starch will be found. If the upper and not the under side is coated there will be no noticeable decrease in the amount of starch formed. The author concludes that under ordinary conditions the main gas exchange is through the stomata and that through the cuticularized epidermis is very small in comparison. This is true even in very young leaves. When the cuticularized surface is scratched or cut, allowing the entrance of CO_2, starch will be formed by the cells receiving CO_2 through the wound. When the air around the leaves contains about 5 per cent. CO_2 a sufficient amount passes through the cuticularized walls to make a large amount of starch.

III. *Prejudicial effect of an increased amount of salt in the substratum on assimilation.*

Schimper in his work on "the Indo-Malayan strand-flora"[10] pointed out the xerophytic nature of most halophytes or salt marsh plants, and also the fact that the presence of a great amount of salt in the tissue is detrimental to assimilation.

[8]Kreusler, Ueber eine Methode zur Beobachtung der Assimilation und Athmung der Pflanzen und einige diese Vorgänge beeinflussende Momente. Tagebl. der Naturforscher-Versammlung zu Strassburg **1885:** 539.

[9]Brown & Morris, A contribution to the chemistry and physiology of foliage leaves. Jour. Chem. Soc. Transactions London **63:** 604–677. 1893.

[10]Schimper, A. F. W., Botanische Mittheilungen aus den Tropen. Die Indo-Malayische Strandflora. Heft 3. 1891.

He considers the reduced transpiration as a guard against excessive accumulation of salt. Stahl goes on with the work for non-halophytes where Schimper left it. He cultivated corn plants in normal solutions with and without 5 per cent. solution of cooking salt; also plants in soil, some watered with the salt solution and others with well water. In all cases he found that the plants given the salt solution stopped developing in a few days, while control plants kept on developing. It was found that there was no starch or sugar present in the plants watered with salt solution while it was plentiful in the others. In a few hours after watering with salt solution the stomata were closed, and the cobalt test showed very little evaporation, even in sunlight. When these plants with the stomata closed were exposed to an atmosphere containing several per cent. of CO_2 they were able to make starch. The presence of sodium chloride in any part of the plant may be demonstrated (according to Schimper) by the use of a saturated aqueous solution of thallium sulphate on tissues freed from air. Crystals of chlorothallium will appear in cells containing the salt. The crystals are black by reflected light. In this way Stahl found that the sodium chloride passes into the epidermal cells but not into the guard cells of the stomata. This, of course, explains their closing.

Leaves of *Alisma Plantago*, *Menyanthes trifoliata*, and *Lilium candidum* were allowed to absorb thallium sulphate (which does not cause the closing of the stomata for some time at least). After allowing transpiration to go on for some time, the chlorothallium was precipitated with calcium chloride. It appears first and most strongly in the guard cells, showing, as Stahl thinks, that there is a current of water in this direction caused by the loss from the guard cells. Later the salt appears in the other epidermal cells also.

Now in regard to the halophytes, it is well known that they thrive perfectly even with large amounts of salt in their tissues. The reason for this as far as the cells themselves are concerned is not clear. Schimper pointed out the xerophytic nature of the halophytes which have the most heterogeneous arrangements for the reduction of transpiration. Stahl shows, however, that these plants do lose large amounts of water and that all these arrangements for the reduction of transpiration are as far as possible to take the place of the lack of the power to close the stomata, so common in marsh plants. In this case, Stahl considers that the staying open on the part of the stomata has to be accepted as a "necessary evil" in order to grow in a substratum that renders the development of other plants impossible on account of the closing of the stomata due to the salt.

IV. *Closing remarks.*

The closing remarks of the paper are especially interesting because they give more clearly the author's ideas on several important questions.

He points out in the first place that the power to open and close the stomata has been of great value in the evolution of plants, in that it gives them the power to grow in a much wider moisture range. Those which have not this power can grow only in extremely moist places.

He goes on to say that the fact that transpiration represents a usual accompaniment of assimilation has been understood in different ways. Some look upon it as a necessary evil, while others (especially following Sachs) recognize in it an important physiological function, whose significance lies in the fact that it enables a continuous supply of water containing mineral nutriments, to reach the assimilating cells.

The opposite opinion has been expressed in a most emphatic manner by Volkens and is based (in his case) on the astonishingly many sided arrangements for the protection of desert plants against loss of water.

In support of the first idea Stahl states that in numerous domestic and especially tropical plants, various arrangements exist which can be explained in no other way than that they are used in transpiration. Many plants are, of course, able to rid themselves of an excess of water in some other way, as by water pores; but still there are many which have no water pores. Here it is transpiration alone which carries off the water from the leaves and makes room for a fresh supply containing nutritive substances. He thinks the importance of transpiration may also depend on the fact that it promotes the distribution of mineral nutrients. He agrees with Sachs's sentence that "the organization of the land plants is only comprehensible when we bear in mind the indicated purpose of the water stream." While in the desert and prairie plants, there are many water-saving devices, on the other hand, in the shade plants and those growing in the dampest tropical countries there are modifications, according to the author, "that only become comprehensible to us when we sufficiently value the great importance of transpiration."

The first of these is the spongy parenchyma, which is especially fully developed in plants growing in moist, shady places and in the dampest tropical countries. The many branched cells, bordering on large intercellular spaces, facilitate evaporation. *Polypodium setigerum* is a good example of a tropical plant of this kind. It is made up almost entirely of spongy parenchyma, bordering on very large intercellular spaces.

In his article on rain fall and the shape of leaves,[11] Stahl concludes that the principal use of the draining of the leaf surface was to promote transpiration. The author promises another paper containing more complete discussion of the adaptations for transpiration.

In this connection I will call attention to what Dr. H. Schenck[12] has to say in regard to the development of spongy parenchyma in shade and especially in water plants. He does not consider it in any way as a means of facilitating transpiration, but on account of the less intense light it is necessary in order to increase the surface as well as the number of working cells which may be exposed to CO_2. This latter view it seems to me is most probably the true one.

Although Stahl holds firmly to the notion that transpiration is of great physiological value to the plant, yet the facts which he has observed may be readily interpreted the other way. In spite of some uncomplimentary things which have been said about this paper by a foreign reviewer,[13] it is one of the most suggestive and helpful articles on transpiration which has appeared for several years.—ALBERT F. WOODS.

[11] Stahl, E., Regenfall und Blattgestalt. Annales du jardin botanique de Buitenzorg **11**: 98–182. *pl. 5–12.* 1893.

[12] Die Biologie der Wassergewächse 7–9. Bonn, 1886.

[13] Jensen, H., Botan. Litteraturblade —: 149–152. 1894. [Copenhagen.]